

**What is Claimed Is:**

- 1                    1.        A method for detecting in a sample a primary amino acid,  
2        said method comprising:  
3                    contacting said sample with an aminoacyl tRNA synthetase of said primary  
4        amino acid to form a first product; and  
5                    detecting said first product.
- 1                    2.        The method of claim 1, wherein said detecting detects  $PP_1$ .
- 1                    3.        The method of claim 1, wherein said detecting detects an aminoacyl  
2        tRNA synthetase:AA-AMP complex of said primary amino acid.
- 1                    4.        The method of claim 1, wherein said sample comprises a plurality of  
2        primary amino acids.
- 1                    5.        The method of claim 1, wherein said aminoacyl tRNA synthetase is  
2        immobilized on a solid support.
- 1                    6.        The method of claim 1, wherein said primary amino acid is  
2        phenylalanine.
- 1                    7.        The method of claim 1, wherein said primary amino acid is glycine.
- 1                    8.        The method of claim 1, wherein said primary amino acid is aspartic  
2        acid.
- 1                    9.        The method of claim 1, wherein said sample is a biological sample.
- 1                    10.      The method of claim 9, wherein said sample is a blood sample or a  
2        serum sample.
- 1                    11.      The method of claim 1, wherein said sample is generated by N-  
2        terminal or C-terminal digestion of a polypeptide or protein.
- 1                    12.      The method of claim 1, wherein said sample comprises amino acids  
2        released by hydrolysis of the peptide bonds of a protein.
- 1                    13.      The method of claim 1, wherein each of the 20 primary amino acids  
2        can be detected.

1 14. The method of claim 1, wherein said first product is labeled and said  
2 detecting is by means of said label.

1 15. The method of claim 1, wherein said first product is directly detected.

1 16. The method of claim 1, wherein said first product is indirectly  
2 detected.

1 17. The method of claim 4, wherein said contacting further comprises  
2 contacting said plurality of primary amino acids with a plurality of aminoacyl tRNA  
3 synthetases for the primary amino acids.

1 18. The method of claim 17, wherein said contacting is with an aminoacyl  
2 tRNA synthetase for each primary amino acid.

1 19. The method of claim 17, wherein said plurality of amino acyl tRNA  
2 synthetases are spatially resolved.

1 20. The method of claim 17, wherein said plurality of amino acyl tRNA  
2 synthetases are immobilized on a solid support.

1 21. The method of claim 17, wherein said plurality of aminoacyl tRNA  
2 synthetases are each located at a known locus of a spatial array, and wherein said detecting is  
3 according to said known locus.

1 22. The method of claim 21, wherein said second product is labeled and  
2 said detecting is by means of detecting said label.

1 23. The method of claim 17, wherein an array is formed by separately  
2 locating said aminoacyl tRNA synthetases each at a known locus of a solid support selected  
3 from the group consisting of microtiter surface, microwell, microchannel and microcapillary  
4 array.

1 24. The method of claim 17, wherein all twenty primary amino acids can  
2 be detected.

1 25. The method of claim 1, wherein said first product is said aminoacyl  
2 tRNA synthetase:AA-AMP complex and said detecting is by indirect means comprising:

3                   contacting said first product with a tRNA for said primary amino acid to form  
4 a second product; and

5                   detecting said second product.

1                   26.     The method of claim 25, wherein said second product is an aminoacyl  
2 tRNA.

1                   27.     The method of claim 25 wherein said second product is AMP.

1                   28.     The method of claim 25, further comprising:  
2                   contacting said first product with a plurality of tRNAs, wherein said  
3 plurality of tRNAs are spatially separated each at a known locus on an array and said  
4 detecting is by contacting said first product with said spatially separated tRNAs to form a  
5 second product; and  
6                   detecting said second product and identifying the detected amino acid  
7 according to said known location of said second product.

1                   29.     The method of claim 25, wherein said tRNA is immobilized on a solid  
2 support and said second product is immobilized on said solid support.

1                   30.     The method of claim 26, wherein said tRNA for said primary amino  
2 acid is fluorescently labeled and said label is used to detect said second product.

1                   31.     The method of claim 26, further comprising contacting said aminoacyl  
2 tRNA with an elongation factor to form a ternary complex and detecting said ternary  
3 complex.

1                   32.     The method of claim 31, wherein said factor is elongation factor Tu or  
2 elongation factor 1A in a complex with GTP or a GTP analog.

1                   33.     The method of claim 32, wherein said GTP analog is a  
2 nonhydrolyzable analog of GTP which is incorporated into said ternary complex.

1                   34.     The method of claim 25, wherein said tRNA is fluorescently labeled  
2 and said label is used to detect the second product.

1                   35.     The method of claim 31, wherein said elongation factor is labeled.

1                   36.     The method of claim 25, wherein an array of tRNAs for the primary  
2 amino acids is formed by separately locating each of said plurality of tRNAs at a known  
3 locus on a solid support.

1                   37.     The method of claim 36, wherein said solid support is selected from  
2 the group consisting of microtiter surface, microwell, microchannel and microcapillary array.

1                   38.     The method of claim 1, wherein said detecting is by means of a  
2 fluorescence detector, a proximity scintillation surface, a spectrophotometer, a luminometer,  
3 a scintillation counter, a Raman spectrophotometer, a charge coupled device camera or a  
4 gamma counter.

1                   39.     The method of claim 1, wherein a molecular sieve through which  
2 compounds of greater than about 6 kDa cannot pass separates said sample from said  
3 aminoacyl tRNA synthetase.

1                   40.     The method of claim 25, wherein all twenty primary amino acids are  
2 detected.

1                   41.     The method of claim 31, further comprising contacting said ternary  
2 complex with a biorecognition element; and detecting the interaction of said ternary complex  
3 with said biorecognition element.

1                   42.     The method of claim 31, wherein each of said tRNA for a primary  
2 amino acid comprises a unique distinguishing label for detection.

1                   43.     The method of claim 31, wherein detecting of said ternary complex is  
2 by means of a biosensor selected from the group consisting of a piezoelectric crystal, a  
3 surface plasmon resonance system, an acoustic wave sensor device, a fluorescence detector or  
4 a proximity scintillation surface.

1                   44.     The method of claim 31, wherein said biorecognition element is bound  
2 to a transducer to create an amino acid biosensor.

1                   45.     The method of claim 31, wherein said elongation factor is immobilized  
2 on said amino acid biosensor.

1                   46.     The method of claim 31, wherein the biorecognition element is a  
2     ternary complex probe immobilized on a transducer.

1                   47.     The method of claim 46, wherein the transducer is an optical fiber, an  
2     electrode, a piezoelectric crystal, a thermistor or a planar wave guide.

1                   48.     The method of claim 31, wherein said tRNA for said primary amino  
2     acid is labeled with a detectable tag.

1                   49.     The method of claim 48, wherein said detectable tag is a fluorophore, a  
2     chromophore, a nanoparticle, a metal, an enzyme, a liposome-based label, an electrogenic  
3     label, ferrocene, biotin or a radioisotope.

1                   50.     The method of claim 31, wherein said elongation factor is labeled with  
2     a detectable tag.

1                   51.     The method of claim 3, wherein said ternary complex is detected using  
2     a ternary complex probe.

1                   52.     The method of claim 51, wherein said ternary complex probe is an  
2     antibody or an antibody fragment specific for said ternary complex.

1                   53.     The method of claim 43, wherein said ternary complex probe is a  
2     nucleic acid.

1                   54.     The method of claim 25, wherein said tRNA for said primary amino  
2     acid is labeled with a fluorophore, a chromophore, a nanoparticle, a metal, an enzyme, a  
3     liposome-based label, an electrogenic label, ferrocene, biotin or a radioisotope.

1                   55.     The method of claim 54, wherein said tRNA is detected by fluorescence,  
2     chromophore, radioactive decay, an electrical signal, or chemiluminescence.

1                   56.     A spatial array for the detection of a primary amino acid in a sample,  
2     wherein said array comprises:  
3                         spatially separated aminoacyl tRNA synthetases or spatially separated  
4     tRNAs for a plurality of the primary amino acids each at a known locus on said array;

means for contacting said sample with said spatially separated synthetases to form a first product.

57. The spatial array of claim 56, wherein said first product is selected from the group consisting of an aminoacyl tRNA synthetase:AA-AMP of said primary amino acid, PP<sub>i</sub>, the aminoacyl tRNA of said primary amino acid, or AMP.

58. The spatial array of claim 56, wherein said spatially separated aminoacyl tRNA synthetases or spatially separated tRNAs collectively provide an aminoacyl tRNA synthetase or tRNA for each of the primary amino acids.

59. The spatial array of claim 58, wherein said spatially separated aminoacyl tRNA synthetases or said spatially separated tRNAs are immobilized at a known locus on said array.

60. The spatial array of claim 58, wherein the spatially separated aminoacyl tRNA synthetase or said spatially separated tRNA for said primary amino acid is labeled.

61. A miniaturized integrated microfluidic amino acid analysis system for performing amino acid analysis, said system comprising

(a) a plurality of reaction microchannels, wherein each microchannel is formed in a planar substrate and wherein each microchannel has a sample inlet for receiving a sample and a discharge outlet,

(b) at least one reservoir for input to said reaction microchannels, wherein said reservoir is in fluid connection to at least one reaction microchannel;

(c) a means for inputting fluid from the reservoir to each reaction channel;

(e) a waste reservoir in fluid connection with said discharge outlet;

(f) a detection system in each of reaction microchannels, said detection system detecting a product of an aminoacyl tRNA synthetase reaction in the microchannels during use of the system;

(g) a main flow channel in fluid connection to said reaction channels joining at said sample inlets, said main flow channel being in fluid connection to one or more reservoirs and having a continuous flow mixer; and

(h) a means for transporting fluid from said reservoir(s) through the main channel and through the reaction channels.

1                   62.     The system of claim 61, comprising a digestion chamber, wherein said  
2 digestion chamber is in fluid communication with said reaction microchannels via said  
3 sample inputs of said reaction microchannels.

1                   63.     The system of claim 61, further comprising a molecular sieve upstream  
2 of said sample inlets of said reaction microchannels.

1                   64.     The system of claim 63, wherein said molecular sieve is a  
2 microdialysis probe or a selectively permeable membrane through which molecules less than  
3 about 6 kDa pass freely.

1                   65.     The system of claim 61, further comprising a syringe interface in fluid  
2 connection to said main channel.

1                   66.     The system of claim 62, wherein said system further comprises a  
2 molecular sieve between said digestion chamber and said reaction microchannel, and a means  
3 for moving fluid from a reservoir or syringe through said molecular sieve and into said main  
4 channel.

1                   67.     The system of claim 61, wherein said said sample inlet of said reaction  
2 chamber is a syringe port.

1                   68.     The system of claim 62, wherein said digestion chamber comprises at  
2 least one exopeptidase and wherein each reaction microchannel comprises at least one  
3 aminoacyl tRNA synthetase and at least one tRNA cognate to the aminoacyl tRNA  
4 synthetase.

1                   69.     The system of claim 61, having a plurality of reaction microchannels,  
2 wherein each reaction microchannel comprises an affinity zone having immobilized  
3 biomolecular recognition molecules, wherein each reaction microchannel is integrated with a  
4 detector system to detect a signal from an AA-tRNA bound to said biomolecular recognition  
5 molecules immobilized in the affinity zone.

1                   70.     The system of claim 69, wherein the signal detected is a mass change,  
2 fluorescence, chromophore, radioactive decay, an electrical signal or chemiluminescence.

1                   71.     The system of claim 69, wherein said detection system comprises a  
2 biosensor selected from the group consisting of a piezoelectric crystal, a surface plasmon  
3 resonance system, an acoustic wave sensor device, a fluorescence detector or a proximity  
4 scintillation surface.

1                   72.     The system of claim 63 further comprising a digestion chamber within  
2 which is at least one immobilized protease, wherein said molecular sieve is between the  
3 digestion chamber and the sample inlet of the reaction microchannels.

1                   73.     The system of claim 61 wherein each reaction channel is integrated  
2 with a detection system for characterizing the properties of the sample, and wherein each  
3 reaction channel is integrated with computer controlled valves that control passage of fluids  
4 from the digestion chamber into each reaction channel.

1                   74.     The system of claim 61 wherein each reaction channel comprises an  
2 aminoacyl tRNA synthetase immobilized on an interior surface of the channel.

1                   75.     The system of claim 74 wherein each reaction channel further  
2 comprises a biomolecular recognition molecule immobilized to a detection region of said  
3 microchannel.

1                   76.     A microtiter plate kit for detecting a primary amino acid in a sample,  
2 said kit comprising:  
3                   (a)     a microtiter plate, said plate having a multiplicity of wells;  
4                   (b)     a plurality of aminoacyl-tRNA synthetases, wherein at least one of said  
5 amino acyl tRNA synthetases is for said primary amino acid;  
6                   (c)     a plurality of tRNAs wherein at least one tRNA is specific for said  
7 primary amino acid;  
8                   (d)     reagents for following the reactions catalyzed by the aminoacyl tRNA  
9 synthetases.

1                   77.     The kit of claim 76, said kit comprising a first set of twenty containers,  
2 wherein each container contains a tRNA specific for a different one of the 20 primary protein  
3 amino acids and a second set of twenty containers wherein each container contains an  
4 aminoacyl tRNA synthetase for a different one of the twenty primary amino acids.



1 78. The kit of claim 76, wherein said tRNA for said primary amino acid is  
2 labeled.

1 79. The kit of claim 78, further comprising an elongation factor for  
2 complexing the aminoacyl tRNA.

1 80 The kit of claim 70, wherein said elongation factor is labeled.

1 81. The kit of claim 78, wherein said elongation factor is Ef-Tu:GTP.

1 82. The kit of claim 80, wherein the elongation factor is labeled with a  
2 fluorophore, a chromophore, a chemiluminescent molecule, an enzyme, a metal, a  
3 nanoparticle or a radioisotope.

1 83. The kit of claim 76, further comprising each of the primary amino  
2 acids.

1 84. The kit of claim 79, further comprising a nonhydrolyzable analog of  
2 GTP.

1 85. The kit of claim 84, wherein said analog is GDPNP.

1 86. The kit of claim 76, further comprising reagents for monitoring the  
2 formation of PPi.

1 87. The kit of claim 76, further comprising reagents for monitoring the  
2 formation of AMP.

1 88. The kit of claim 78, further comprising a ternary complex probe.

1 89. The kit of claim 79, wherein said elongation factor is immobilized to  
2 scintillation proximity assay beads and radiolabeled AA-tRNAs.

1 90. The system of claim 61, wherein biorecognition elements are arrayed  
2 into microchannels.

1 91. The system of claim 61, wherein biorecognition elements are arrayed  
2 into microcapillaries.

1                    92.     The system of claim 61, wherein biorecognition elements are arrayed  
2 to the bottom of flow channels.

1                    93.     The method of claim 32 wherein biorecognition elements are arrayed  
2 on a film or scintillator sheet.

1                    94.     The method of claim 32, wherein the formation of the ternary complex  
2 employs dual distinguishable fluorescent labels, wherein said elongation factor is labeled  
3 with one detectable label and said tRNA for said primary amino acid is labeled with a second  
4 detectable label.

1                    95.     The method of claim 94, wherein said first label is Texas Red and said  
2 second label is fluorescein, and after formation of said ternary complex, the ratio of bound  
3 fluorescein and Texas Red labels is determined using a dual-channel laser scanning confocal  
4 microscope as a detection system.

1                    96.     The method of claim 26, further comprising contacting said aminoacyl  
2 tRNA with an aptamer to form a ternary complex and detecting said ternary complex.

1                    97.     The kit according to claim 76, further comprising an electrochemical  
2 biosensor.

1                    98.     The kit according to claim 76, further comprising an enzyme  
2 biosensor.

1                    99.     The kit according to claim 76, further comprising a biorecognition  
2 element for the ternary complex and an elongation factor.

1                    100. The kit according to claim 99, wherein at least one of said tRNA for said  
2 primary amino acid or said aminoacyl tRNA synthetase for said primary amino acid, or said  
3 biorecognition element for said ternary complex, or said elongation factor is immobilized to a  
4 solid support.

1                    101. The kit according to claim 99, wherein at least one of said tRNA for  
2 said primary amino acid, said aminoacyl tRNA synthetase for said primary amino acid, said  
3 biorecognition element for the ternary complex, or said elongation factor is labeled.

1                    102.    The kit according to claim 101, wherein said label is selected from the  
2    group consisting of a fluorescent label, a radiolabel, a colorimetric label, and an enzyme  
3    label.